nine chondrocytes. By looking at cartilage proteins expression and degradative enzymes activity, we hope to identify GDF-5 action on OA cartilage homeostasis.

Methods: Human OA chondrocytes were isolated from patients undergoing knee replacement. Canine OA chondrocytes were generated from the Nuki-Pond dog model. After in vitro expansion, chondrocytes were co-incubated with increasing concentrations of rhGDF-5. Expression of selected markers of cartilage metabolism (aggrecans, type-I and -II collagen, ADAMTS-4 and -5) was analyzed at the mRNA and protein levels using qRT-PCR and western blot respectively. Aggrecans degradation products, which reflect the activity of aggrecanases, were also analyzed by western blot following GDF-5 stimulation.

Results: Similar responses were recorded in human and canine chondrocytes. Stimulation of human and canine OA chondrocytes with rhGDF-5 led to a dose-dependent increase of aggrecans and type II/type I collagen ratio, with a concomitant decrease in both ADAMTS-4 and ADAMTS-5 mRNA expression. A similar action for rhGDF-5 was observed for aggrecans and ADAMTS-4 and ADAMTS-5 at the protein level. Finally, aggrecans degradation products were also significantly decreased in OA chondrocytes following rhGDF-5 stimulation.

Conclusions: Interestingly, the addition of rhGDF-5 had a dual effect on OA chondrocytes, increasing markers of anabolic activity and decreasing catabolic enzymes expression and activity. These results suggest a key role for GDF-5 in maintaining cartilage homeostasis, an action that could be lost in OA. Because GDF-5 concentrations are constant in normal and OA cartilage, interference with GDF-5 signalling may occur in OA, an anomaly that appears to be recovered by the addition of rhGDF-5. Further in vivo studies will evaluate the potential of GDF-5 as a disease modifying drug in OA.

234

INTERACTION BETWEEN 4-HYDROXYNONENAL-MODIFIED EXTRACELLULAR MATRIX AND OSTEOARTHRITIC CHONDROCYTES OR OSTEOBLASTS INDUCES CHANGES IN CELL PHENOTYPE AND FUNCTION

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Purpose: Extracellular matrix (ECM) degradation and formation are closely linked and tightly regulated in order to maintain cartilage and bone homeostasis. In cartilage and bone, accumulation of 4-hydroxynonenal (HNE), a product of lipid peroxidation (LPO), undergoes considerable alterations in ECM structure and thereby rendering it more prone to mechanical damage. This study aimed to demonstrate that interaction between HNE-modified extracellular matrix and chondrocytes or osteoblasts induced changes in cell morphology and function of these cells.

Methods: Plates culture were coated with 0.1 mg/well type II collagen (Col II) and then modified by increasing concentrations of HNE (0.1 to 2 mM). Chondrocytes or osteoblasts where then incubated for 48 hrs in appropriate culture medium. Cell viability was evaluated by MTT test. The protein expression of adhesion molecules such as integrin αV/β3 and inter-cellular adhesion molecule-1 (ICAM-1) is performed by Western blot. The level of matrix metalloproteinase-13 (MMP-13), prostaglandin 2 (PGE2) and osteocalcin (OC) was determined by ELISA using commercial kits. Alkaline phosphatase (ALPase) activity was measured as the release of p-nitrophenol hydrolysed from p-nitrophenyl phosphate.

Results: After 48 hrs of incubation, the modification of Col II with 2 mM HNE induces shape change of chondrocytes and osteoblasts from elongated phenotype to rounded phenotype with a 10% decrease in cell viability. At a functional level, the modification of Col II with 0.1-0.5 mM HNE enhanced the production of integrin αVβ3, ICAM-1, MMP-13 and PGE2 in chondrocytes, and OC release and ALPase activity in osteoblasts. In contrast, the modification of Col II with 1 and 2 mM HNE reduced their production in both cell types.

Conclusions: Our results showed that interaction between HNE-modified Col II and chondrocytes or osteoblasts induced changes in cell phenotype of chondrocytes/osteoblasts and modulate the expression of catabolic and inflammatory factors. As described previously by our laboratory, we suggest that accumulation of HNE/Col II adducts in OA cartilage and bone may play a critical role in OA pathogenesis.

235

DIFFERENTIAL RESPONSE OF CARTILAGE EXPLANTS TO CLINICALLY-RELEVANT LEVELS OF IL-1 AND FIBRONECTIN FRAGMENTS

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Purpose: In vitro, it is known that high doses of recombinant interleukin-1 (IL-1) causes rapid and extensive cartilage degradation characterized by loss of proteoglycan from tissue to surrounding culture medium. However, IL-1 is only one of several potentially catabolic factors detected in synovial fluid from osteoarthritic (OA) joints, and has been shown to be present at much lower concentrations than those typically employed in vitro. Our goal was to expose cartilage explants to levels of IL-1 similar to those we measured in OA synovial fluid, assess the loss of sulfated GAG from the tissue, and determine the effect of IL-1 blockade using IL-1 receptor antagonist (IL-1ra).

Methods: Levels of IL-1 were determined in synovial fluid collected from OA patients. Bovine articular cartilage was harvested from the patellofemoral groove of 3 adult animals, and prepared as full-thickness discs 6 mm in diameter. These cartilage explants were equilibrated for 48 hours in medium (DMEM with 50 μg/mL gentamicin), then transferred to culture wells containing fresh medium supplemented with IL-1 alpha ranging from 5000 ng/mL (often used for in vitro degradation studies) to more clinically-relevant levels (200, 20, 16, 8, 4, 2, 1, 0.5, and 0 ng/mL) on measured levels from above. To assess the contribution of other potential catabolic factors, some cultures were also supplemented with 30 kDa N-terminal fragment of fibrinectin (Fn-f) at reported physiologic concentration (0.8 μM). The effect of IL-1 blockade was assessed by preincubating some cartilage samples with 1.4 nM IL-1ra for 2 hours before addition of IL-1 or Fn-f. After 4 days, culture media were collected and assayed for sulfated GAG content by DMMB, normalized to initial tissue wet weight. GAG distribution was also visualized in cartilage discs by safranin-O staining.

Results: Incubation of cartilage with IL-1 gave a dose-dependent decrease in sGAG release between 5000 and 20 pg/mL. The GAG content of the culture medium was not different from control explants (no IL-1) for the range 0.5-20 pg/mL IL-1. Similarly, there was no difference in safranin-O histology in this dose range. Addition of IL-1ra to the culture medium completely inhibited this loss. However, when Fn-f was included in the culture medium, GAG release was increased, and the additional degradation was not able to be inhibited by IL-1ra.

Conclusions: In later-stage OA patients, we and others have detected IL-1 at very low levels. Incubation of cartilage with IL-1 at these concentrations did not induce GAG release over that of untreated cartilage explants. However, at least one other catabolic factor, Fn-f, induced degradation at a more clinically-relevant concentration. It may be that in advanced stages of OA, the contribution of IL-1 to degradation is not as pronounced as that of other catabolic factors. While IL-1 may be more involved in earlier OA,